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Immunogens of Pasteurella

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ABSTRACT

The family Pasteurellaceae Pohl contains Gram-negative, facultatively anaerobic and fermentative bacteria of the genera *Pasteurella, Haemophilus,* and *Actinobacillus*. Approximately 20 different species of the genus *Pasteurella* have been identified using phenotypic and genetic analyses. Of these species, *P. multocida* and *P. haemolytica* are the most prominent pathogens in domestic animals causing severe diseases and major economic losses in the cattle, swine, sheep, and poultry industries. Mechanisms of immunity to these bacteria have been difficult to determine, and efficacious vaccines have been a challenge to develop and evaluate.

Pasteurella multocida of serogroups A and D are mainly responsible for disease in North American poultry and pigs and to a lesser extent in cattle. Fowl cholera in chickens and turkeys is caused by various serotypes of *P. multocida* serogroup A and characterized by acute septicemia and fibrinous pneumonia or chronic fibrinopurulent inflammation of various tissues. Current biologicals in use are live *P. multocida* vaccines and bacterins. Potency tests for avian *P. multocida* biologicals are a bacterial colony count for vaccines and vaccination and challenge of birds for bacterins. Somatic antigens, particularly lipopolysaccharide (LPS), appear to be of major importance in immunity. In North American cattle, *P. multocida* serogroup A is associated mainly with bronchopneumonia (enzootic pneumonia) in young calves; however, it is occasionally isolated from fibrinous pleuropneumonia of feedlot cattle (shipping fever). Biologicals currently available are modified-live vaccines and bacterins. The potency test for vaccines is bacterial colony counts. The test for bacterin potency is vaccination and challenge of mice. Important immunogens have not been well characterized for *P. multocida* infection in cattle. In swine, *P. multocida* infection is sometimes associated with pneumonia; however, its major importance is in atrophic rhinitis. A protein toxin (dermonecrotic toxin), produced by toxigenic strains of *P. multocida* types A and D, and concurrent infection with *Bordetella bronchiseptica* appear to be the major factors in development of atrophic rhinitis. Currently available biologicals are bacterins and inactivated toxins (toxoids). The toxin appears to be the major immunogen for preventing atrophic rhinitis. There are, however, no standardized requirements for potency testing of *P. multocida* type D toxoid.

Various serotypes of *P. haemolytica* biotype A are responsible for severe fibrinous pleuropneumonia of cattle and sheep, occasionally septicemia of lambs, and mastitis in ewes. Several serotypes of *P. haemolytica* biotype T are isolated from acute septicemia of lambs. The currently available *P. haemolytica* biologicals are modified-live vaccines, bacterins, bacterial surface extracts, and culture supernates that contain an exotoxin (leukotoxin). Most biologicals contain *P. haemolytica* biotype A serotype 1; however, biotype T serotypes 3 and 4 are occasionally included. As with *P. multocida* vaccines, the potency test for a *P. haemolytica* vaccine is a bacterial colony count. There are no stan-

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dard guidelines for potency tests for *P. haemolytica* bacterins or extracts and supernate biologicals. The major immunogens for *P. haemolytica* appear to be the leukotoxin, capsule, outer membrane proteins, and iron-regulated proteins.

INTRODUCTION

The family *Pasteurellaceae* Pohl contains Gram-negative, facultatively anaerobic and fermentative bacteria of the genera *Haemophilus, Actinobacillus* and *Pasteurella*. Members of the genus *Pasteurella* cause septicemia, respiratory disease, and mastitis in numerous domestic animal species (Confer et al., 1990). *Pasteurella* spp. have been associated with disease and attempts to develop efficacious biologicals for this group of bacteria date back to the time of Pasteur; however, development of such vaccines has been a formidable task.

A complete description of the taxonomy of the genus *Pasteurella* is beyond the scope of this review (Mutters et al., 1989). However, a brief review of several key points is warranted. As described in *Bergey's Manual of Systematic Bacteriology* (Carter, 1984), the genus *Pasteurella* can be divided among six species based on beta hemolysis, growth on MacConkey's agar, indole production, urease activity, gas from carbohydrates, and acid production from lactose or mannitol. These species are *P. multocida, P. haemolytica, P. pneumotropica, P. ureae, P. aerogenes,* and *P. gallinarum*. Phenotypic properties, however, only account for expression of 10–20% of the genome, and molecular techniques offer more critical analysis of relatedness among species. Therefore, the *Pasteurellae* have been examined using such techniques as DNA/DNA hybridization (Mutters et al., 1986), 2-dimensional protein electrophoresis (Olsen et al., 1987), and rRNA analysis (Dewhirst et al., 1992). Such analyses have resulted in reclassification of several *Pasteurella* species into 11 species in the genus *Pasteurella sensu stricto,* which can be differentiated using certain common phenotypic properties beyond those used in Bergey's manual (Mutter et al., 1989). One of the major organisms affected in this classification is *P. multocida,* which is no longer classified merely as a single bacterial species isolated from a wide variety of diseases in several animal species. Appropriate subspecies of *P. multocida* are now recognized, and these tend to be relatively animal species specific. Several traditional *Pasteurella* species, including *P. haemolytica,* were excluded from *P. sensu stricto,* and their classifications as *Pasteurella* remain questionable especially in light of recent rRNA analyses (Dewhirst et al., 1992). In this review, I will concentrate on immunogens of *P. multocida* and *P. haemolytica* (as traditionally classified) as they relate to development of efficacious biologicals for control of domestic livestock in North America.

Pasteurella multocida

Antigenic typing

P. multocida is classified by capsular (serogroup) and somatic (serotype) antigen typing (reviewed in Rimler and Rhoades, 1989). Encapsulated *P. multocida* can be separated into five serogroups (A, B, D, E, and F) by passive hemagglutination of erythrocytes coated with crude cell extracts containing capsule specific antigen which includes lipopolysaccharide (LPS) and non-antigenic polysaccharide (Carter, 1968). Somatic serotyping of *P. multocida* is most commonly done by gel diffusion precipitin tests (Heddleston et al., 1972). This system recognizes sixteen serotypes and uses chicken antisera made against *P. multocida* bacterins reacted against heat-stable antigens from formalinized-saline suspended bacteria. Specific somatic antisera can be obtained from chickens immunized with purified LPS (Rimler, 1984).

Immunogens

Purified *P. multocida* capsule behaves as a hapten when injected in most animals and has not been extensively investigated as an immunogen against diseases in North America livestock. *P. multocida* LPSs have similar chemical and biological properties to the R-type LPSs of other gram negative bacteria (Lugtenberg et al., 1984). Purified *P. multocida* LPS is antigenic; however, the extent of antibody response following immunization depends on animal species inoculated, LPS type used, and route and method of inoculation (Rimler and Phillips, 1986). In addition, protection afforded by immunization with *P. multocida* LPS is somewhat animal species dependent. In general, *P. multocida* LPS seems to be a major immunogen in birds (Rhoades and Rimler, 1989). However, Tsuji and Matsumoto (1988) suggested that a LPS-protein complex is essential for induction of immunity against *P. multocida* infection in turkeys. The role of LPS as an immunogen in mammals remains controversial. Mice, cattle, and rabbits have not been readily protected against *P. multocida* infection following immunization with LPS (Rimler and Rhoades, 1989). Recently, Wijewardana et al. (1990) found that a bactericidal monoclonal antibody (mAb) against LPS completely protected mice against homologous challenge with live *P. multocida*. However, monoclonal antibodies to LPS that were opsonic but not bactericidal only partial protected mice against *P. multocida* challenge (Ramdani and Adler, 1991). Lu et al. (1991 b) failed to protect mice against *P. multocida* infection by passive transfer of affinity-purified rabbit anti-LPS serum.

Recently, outer membrane proteins (OMPs) of *P. multocida* have been studied as potential immunogens. The immunogenic role of these proteins has been suspected for years; however, LPS and capsular contamination of OMPs have limited interpretation of their immunogenic potential. Lugtenberg et al. (1984) demonstrated three envelope protein profiles of *P. multo-*

cida isolates from swine. Profiles differed primarily on the migration of a surface protein (protein H) with molecular mass of approximately 36–38 kDa (Lugtenberg et al., 1986). Knights et al. (1990) isolated outer membranes of the various serogroups of *P. multocida* and demonstrated that their electrophoretic patterns were markedly different from those of *P. haemolytica*. Iron-regulated OMPs were demonstrated in *P. multocida* and appear to be siderophore receptors (Ikeda and Hirsh, 1988; Choi-Kim et al., 1991). Production of those proteins in vivo was suggested by their demonstration with convalescent sera from *P. multocida*-infected turkeys.

The immunogenic role of *P. multocida* OMPs has been best characterized in rabbits. Lu et al. (1988) demonstrated that rabbits mounted major antibody responses against 5 *P. multocida* OMPs (27, 37.5, 49.5, 58.7, and 64.4 kDa). They further demonstrated that vaccination with *P. multocida* outer membranes protected rabbits against homologous challenge (Lu et al., 1991 c), and protection seemed to be due to antibodies against OMPs and not LPS (Lu et al., 1991 b). More specifically, a mAb against a 37.5 kDa OMP protected both mice and rabbits against *P. multocida* challenge (Lu et al., 1991a). Additionally, Truscott and Hirsh (1988) demonstrated a 50 kDa *P. multocida* OMP that was antiphagocytic. Turkeys given antibodies specific for that OMP were protected against challenge. Abdullahi et al. (1990), however, failed to demonstrate in *P. multocida*-challenged mice any correlation between protection and antibody response to OMP from bovine isolates of *P. multocida*. Membrane-associated cross-protection factor(s) have been identified in *P. multocida* harvested from the blood of turkeys dying of experimental fowl cholera (Rimler and Rhoades, 1989b). These factors stimulate heterotypic immunity to *P. multocida*. Finally, the immunogenic role of iron-regulated *P. multocida* OMP has not been investigated.

Certain *P. multocida* isolates of the A and D serogroups produce antigenically similar protein toxins (approximately 145 kDa) that are toxic for bovine embryonic lung and Vero cells in vitro, are lethal for rodents and birds, induce osteolysis of swine turbinate bones, and produce hemorrhage and necrosis when injected into guinea pig skin (Rimler and Brogden, 1986; Rimler and Rhodes, 1989). Because of the latter effect, the toxin was called dermonecrotic toxin; however, the term *P. multocida* toxin (PMT) is in current use. Although PMT has many characteristics of an exotoxin, it is not secreted from living intact *P. multocida* (Dali et al., 1991), but must be extracted from bacteria by sonication.

PMT has been demonstrated in *P. multocida* isolates from various animal species; however, it is mainly a virulence factor in atrophic rhinitis of pigs. As an immunogen, inactivated PMT (toxoid) induces protection against the lethal effects of PMT in rats and mice (Thurston et al., 1991) and against experimental atrophic rhinitis in pigs (Foged et al., 1989). MAbs against PMT can neutralize its lethal effects in mice (Foged, 1988). The gene for PMT has

been cloned and expressed in *Escherichia coli* and characterized (Petersen and Foged, 1989). A deletion mutant of PMT, which is deficient in 121 amino acids in the amino-terminal quarter of PMT, had markedly reduced toxicity for cell culture, mice, and guinea pig skin; however, it retained its immunogenicity for mice and gilts (Nielsen et al., 1991; Petersen et al., 1991). In those studies, vaccinated mice were protected against the lethal effects of PMT, and pigs born to vaccinated gilts had enhanced resistance against experimental atrophic rhinitis.

Poultry biologicals

Various somatic types and serogroups of *P. multocida* especially serogroup A, serotypes 1, 3, and 4, are recognized as the primary cause of fowl cholera in chickens and turkeys (Rhoades and Rimler, 1989). Disease may manifest as an acute septicemia characterized by disseminated intravascular coagulation, petechial to ecchymotic hemorrhages, multifocal hepatic and splenic necrosis, and fibrinous pneumonia. Chronic fowl cholera occurs as localized fibrinopurulent exudate and necrosis in a variety of locations including sinuses, air sacs, lungs, wattles, foot pads, and bones and joints. *P. multocida* factors that may be important for virulence are LPS (Rimler et al., 1984), capsule (Tsuji and Matsumoto, 1989), plasmids, and resistance to complement-mediated bacteriolysis (Lee et al., 1991).

Biologicals for immunization of poultry against *P. multocida* are currently of two types: bacterins and attenuated vaccines. Bacterins induce somatic type-specific immunity, whereas vaccines confer some degree of cross-serotypic immunity. *P. multocida* bacterins are potency tested in chickens or turkeys using a two-stage test. Stage one uses twice-vaccinated and unvaccinated birds challenged intramuscularly with an appropriate virulent reference serotype not less than 14 days after the last vaccination. If 8 or more unvaccinated birds die, then the test is valid and results are evaluated as in Table 1. Stage 2 is conducted like stage 1 and evaluated as in Table 1. Potency test for the avian *P. multocida* vaccine is an arithmetic mean count of colony forming units (CFU) of bacteria. Vaccines must contain CFUs greater than in the test vaccine used in an immunogenicity study conducted similar to the bacterin

Table 1
Potency test evaluation for avian *Pasteurella multocida* bacterins

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of dead vaccinates for	
			Satisfactory serial	Unsatisfactory
1	20	20	⩽6	⩾9
2	20	40	⩽15	⩾16

potency tests. During the expiration period, the final vaccine samples must contain CFUs at least two-times greater than that used to immunize birds in the immunogenicity test.

The major *P. multocida* immunogen in birds appears to be LPS based on the ability to immunize birds against disease using extracted LPS (Rhoades and Rimler, 1989). However, the role of non-LPS antigens in stimulating immunity to avian *P. multocida* isolates is not known. Several attempts have been made to correlate antibody responses to *P. multocida* with resistance to challenge. Avakian et al. (1986,1989) showed positive correlation between antibody responses to capsular and KSCN-extracted *P. multocida* antigens and resistance of broilers to challenge but showed no correlation between protection and antibody titers to sonicated bacteria. Schlink and Olson (1989) demonstrated correlations between high antibody titers in a microagglutination test and survival of infected turkeys. Determination of the immunogenic role of non-LPS such as capsule, OMP, and in vivo expressed antigens (Glisson and Cheng, 1991) such as the membrane-associated cross-protection factor(s) are critical to understanding immunity to fowl cholera.

Cattle biologicals

P. multocida serogroups B and E are associated with hemorrhagic septicemia (Carter and De Alwis, 1989), whereas respiratory disease is mainly associated with serogroup A (Frank, 1989). Hemorrhagic septicemia is not an important disease in the United States and will not be considered further in this review. *P. multocida* serogroup A is most commonly associated with a fibrinous bronchopneumonia that is less fulminating than the fibrinous pleuropneumonia associated with *P. haemolytica* infection (Dungworth, 1985). *P. multocida* can be isolated from bronchopneumonia in feedlot cattle or from enzootic pneumonia of calves less than 6 months old.

Biologicals available for *P. multocida* in cattle are bacterins and vaccines, usually in combination with *P. haemolytica*. The potency test for non-avian *P. multocida* bacterins is vaccination and challenge of mice. An acceptable potency for mice is 1/20 of the least dose recommended for other animals. The test bacterin is tested against a standard bacterin using at least three five-fold dilutions. Each bacterin dilution is tested in 20 mice by 2 intraperitoneal injections 14 days apart. Mice are challenged intraperitoneally 10–12 days after the second vaccination with 100–10 000 LD_{50} of virulent *P. multocida*. The relative potency (RP) of the test bacterin is calculated as follows: RP = reciprocal of 50% endpoint dilution (highest dilution protecting 50% of the mice) of test bacterin/reciprocal of 50% endpoint dilution of the standard. If the RP is <0.50, than the bacterin being tested is unsatisfactory. *P. multocida* vaccines are potency tested by determination of CFUs in the vaccine. Standards are set as described for the avian vaccines. The major differ-

ence being that the reference immunogenicity studies are conducted in calves using a respiratory challenge.

Little has been published about *P. multocida* immunogens of importance for protection against bovine respiratory disease. Limited information is available on immunogens of *P. multocida* isolates from hemorrhagic septicemia (Carter and De Alwis, 1989; Dawkins et al., 1991). Capsular antigen (Nagy and Penn, 1976), LPS or LPS-protein complex, and various proteins (Dawkins et al., 1991) have been suggested as important immunogens for *P. multocida* serogroups B and E. Because of the difficulty in protecting mice with *P. multocida* LPS and the similarity between *P. multocida*-induced respiratory disease in cattle and that seen in the rabbit, OMP (Lu et al., 1991a, b, c) should be investigated as potential immunogens for cattle.

Swine biologicals

P. multocida serogroups A and D are associated with fibrinous bronchopneumonia, lymph node abscesses, and atrophic rhinitis in swine (Dungworth, 1985). The major economically important disease is atrophic rhinitis, a disease of young swine characterized by bacterial-induced atrophy of nasal turbinates (Chanter and Rutter, 1989). Turbinate and mucosal lesions result in clinical signs of upper respiratory disease and poor weight gain. When lesions are severe, facial distortions such as a twisted snout may be seen. Severe atrophic rhinitis occurs when the nasal cavity is colonized by large numbers of toxigenic *P. multocida*, particularly serogroup D. Colonization is enhanced by concurrent nasal infection with cytotoxin-producing *Bordetella bronchiseptica* (Chanter, 1990). The PMT induces degeneration and necrosis of osteoblasts with subsequent osteoclastic osteolysis of the turbinate bones, predominately the ventral turbinates (Dungworth, 1985). Epithelial hyperplasia accompanies the bony changes. PMT was not toxic for swine alveolar macrophages in vitro (Pifoan, 1986), and the role of PMT in swine pneumonia is unknown.

Biologicals currently available for *P. multocida* in swine are bacterins with and without PMT, often packaged in conjunction with other bacterins such as *B. bronchiseptica, Erysipelothrix rhusiopathiae,* and *Actinobacillus pleuropneumoniae.* The efficaciousness of *P. multocida* bacterins without PMT in controlling swine pneumonia or atrophic rhinitis is questionable (Chanter and Rutter, 1989). However, immunity to atrophic rhinitis seems to be readily induced passively with PMT-specific antisera (Chanter and Rutter, 1989) and actively with biologicals containing *P. multocida* PMT toxoids (Foged et al., 1989) or deletion mutants of recombinant PMT (Nielsen et al., 1991; Petersen et al., 1991). Currently there is no approved standard potency test for *P. multocida* toxoid vaccines.

PMT is well established as the major immunogen of *P. multocida* in atrophic rhinitis. It is imperative, therefore, that potency tests for PMT biologicals be

developed. To effectively do this will require agreement on a standard model for atrophic rhinitis, standardization of a method of measurement of PMT antigenic mass in a biological preparation, and determination of the required dose of PMT antigen to protect piglets against challenge. The intranasal acetic acid/*P. multocida* challenge of specific-pathogen-free pigs is widely accepted for vaccination/challenge trials (Chanter and Rutter, 1989). Also, Ackermann et al. (1991) recently described a model of atrophic rhinitis using intranasal inoculation of pigs with a sterile sonicate of *B. bronchiseptica* followed by live, toxigenic *P. multocida*. Immunogenicity studies should be conducted using an accepted model, whereby pigs would be vaccinated with varying doses of PMT toxoid whose specific antigenic mass had been quantified using mAbs to PMT in an antigen capturing assay such as an enzyme-linked immunosorbent assay. The minimal antigenic dose required to protect a predetermined number of pigs could be determined and set as the standard for future biologicals. Potency tests would involve the antigenic quantification of PMT toxoid in biologicals and comparison to the standards determined by the immunogenicity study.

Pasteurella haemolytica

Antigenic typing

P. haemolytica can be typed according to capsular antigens into 16 serotypes using indirect hemagglutination or a rapid plate agglutination test (Frank, 1989). Furthermore, *P. haemolytica* isolates can be biotyped as A or T based on colony morphology and carbohydrate fermentation. Recent studies of OMP and the genome of *P. haemolytica* demonstrated marked differences in electrophoretic patterns and nucleic acid sequences between the A and T biotypes (Dewhirst et al., 1992; Knights et al., 1990). *P. haemolytica* biotype T has been reclassified as *Pasteurella trehalosi*. Only *P. haemolytica* biotype A will be considered in this review.

Immunogens

P. haemolytica has numerous potential immunogens. Those with the most potential for stimulating immunity include capsular polysaccharide (Adlam et al., 1984), LPS (Rimsay et al., 1981), OMPs (Squire et al., 1984; Knights et al., 1990), fimbriae (Morch et al., 1987), iron-regulated proteins (Gilmour et al., 1991), and a secreted leukotoxin ([LKT] Shewen and Wilkie, 1985). Two approaches have been used for determining the importance of various immunogens for stimulation of immunity to *P. haemolytica*. First is the vaccination of cattle, sheep, or goats with purified or relatively purified antigens followed by challenge with virulent *P. haemolytica*. The second approach uses sera from cattle previously vaccinated with various biologicals and challenged. The antibody responses to specific antigens are quantitated

and statistically correlated with the lesion score obtained after challenge. Thus, a significant correlation between a high antibody response and resistance to challenge can be used as a predictor of the importance of an antigen in stimulating immunity.

Antibody responses to *P. haemolytica* fimbriae have not been documented. Capsular polysaccharides from five *P. haemolytica* serotypes were purified and characterized (Adlam et al., 1989). *P. haemolytica* A1 capsular polysaccharide is a virulence factor that interferes with phagocytosis and killing of *P. haemolytica* (Czuprynski et al., 1991) and complement-mediated bacteriolysis (Chae et al., 1990). Immunization of ruminants with *P. haemolytica* capsular polysaccharide or live or killed whole cell preparations results in an antibody response to the capsule. Studies in my laboratory indicated that antibody responses to *P. haemolytica* capsular polysaccharide inconsistantly correlated with resistance to experimental challenge in calves vaccinated with various experimental vaccines (Confer et al., 1989). Recently, Conlon and Shewen (1991a) reported that vaccination of calves with purified capsular polysaccharide was ineffective at protecting calves against *P. haemolytica* challenge.

P. haemolytica LPS has classical endotoxin function in vivo (Confer et al., 1990), and it can alter leukocyte function, and is toxic to bovine endothelial cells in vitro (Confer and Simons, 1986a; Paulsen et al., 1989). Antibody responses to the LPS O-antigen are readily detected in calves vaccinated with live and killed *P. haemolytica* biologicals; however, the intensity of antibody responses to LPS did not correlate with resistance to experimental challenge (Confer et al., 1986b). Antibody responses to the toxic lipid A moiety were not demonstrated.

The antibody responses to *P. haemolytica* OMP have been incompletely characterized. In my laboratory, resistance to experimental challenge was enhanced by vaccination with surface extracts of *P. haemolytica* (Confer et al., 1989), and antibody responses to protein antigens in those extracts correlated with resistance. Mosier et al. (1989) showed that high antibody responses to several proteins in the surface extract correlated with resistance to experimental challenge. The highest correlations were for antibody responses to proteins with molecular masses of 86, 66, 49, and 31 kDa. Several of those proteins have molecular masses equivalent to major OMP of *P. haemolytica* (Knights et al., 1990). Recently, Morton et al. (1990) showed that vaccination of cattle with *P. haemolytica* OMP-enriched preparations induced serotypic immunity against experimental challenge.

Iron-regulated proteins of *P. haemolytica* have been described (Deneer and Potter, 1989; Donachie and Gilmour, 1988; Lainson et al., 1991). The 100 and 70 kDa proteins are located in the outer membrane of *P. haemolytica* grown in vivo or under iron-restricted in vitro conditions. Recently, a 35 kDa iron-regulated periplasmic protein was demonstrated in *P. haemolytica* A2

(Lainson et al., 1991). Gilmour et al. (1991) demonstrated that sodium salicylate extract vaccines of *P. haemolytica* A2 prepared from bacteria grown under iron-restricted conditions protected sheep better than similar vaccines prepared from *P. haemolytica* grown in media containing iron.

The *P. haemolytica* LKT has received much acclaim as an immunogen against pneumonic pasteurellosis of cattle. The LKT is a member of the RTX family of toxins (Lo, 1990), is lytic for ruminant leukocytes (Clinkenbeard et al., 1989), and is, therefore, thought to have a major role in the pathogenesis of *P. haemolytica*-induced pneumonia. The genes for LKT have been cloned and sequenced (Lo et al., 1985). Antibodies to LKT are not *P. haemolytica* serotype specific. Vaccination of cattle with live *P. haemolytica* or culture supernates induced high neutralizing antibody titers to LKT, and high titers correlated with resistance to experimental or natural infection with *P. haemolytica* (reviewed in Confer et al., 1988). Shewen and Wilkie (1988) demonstrated that antibodies to surface antigens as well as to LKT were important in inducing protection in experimental pneumonic pasteurellosis. Conlon and Shewen (1991b) recently demonstrated that the addition of recombinant LKT to a supernate vaccine enhanced protection against experimental challenge. In my laboratory and others, however, protection against experimental challenge was seen in cattle vaccinated with *P. haemolytica* biologicals that did not contain LKT and neutralizing antibodies to LKT were not detected (reviewed in Confer et al., 1988).

P. haemolytica biologicals

Various serotypes of *P. haemolytica* biotype A have been isolated from severe fibrinous pleuropneumonia of cattle and sheep and mastitis and septicemia of sheep (Confer et al., 1990) . For the purpose of this review, I will concentrate on the major disease problem associated with *P. haemolytica* in North America, fibrinous pleuropneumonia or shipping fever of cattle. *P. haemolytica* A1 is the most common serotype isolated from shipping fever. This disease is usually a more fulminating and potentially fatal pneumonia than that produced by *P. multocida* infection. Death losses in feedlot and stocker cattle and economic losses to the industries can be severe. The pathogenesis of the *P. haemolytica*-induced pneumonia usually requires stress and/or concurrent viral infection and details have been previously described (Frank, 1989). Although the pathogenesis has not been completely elucidated, LKT, by lysing resident and incoming leukocytes, probably contributes to necrosis seen in the pulmonary alveoli. Endotoxin probably contributes to vascular damage and systemic signs of illness (Confer et al., 1990).

Currently available *P. haemolytica* biologicals are modified-live vaccines, bacterins, bacterial surface extracts, and culture supernates that contain LKT. Most biologicals in the U.S. contain *P. haemolytica* A1; however, *P. haemolytica* T3 and T4 are occasionally included. Currently, potency testing of live

P. haemolytica vaccines is by determination of CFUs. Live *P. haemolytica* vaccines protect cattle well against experimental pneumonic pasteurellosis, but they can have undesirable side effects such as fever, localized abscesses, and lameness (Confer et al., 1986c). Because of these side effects, many of the newer *P. haemolytica* biologicals use the subunit, extract, or supernate approach. There is no current standard for potency tests for non-living *P. haemolytica* biologicals. Development of an acceptable potency test is important for assuring future efficacy.

Two key issues must be addressed in developing *P. haemolytica* potency tests for non-living biologicals, challenge models and critical immunogens. First is determining acceptable challenge models for immunogenicity studies. Results obtained in mouse models in the past, using intraperitoneal vaccination/challenge, had little or no relevance to respiratory disease of cattle. Numerous respiratory challenge models have been used in calves experimentally, and these have been reviewed (Frank, 1989). Those models vary as to the age of cattle used, route of challenge and method of challenge delivery, whether calves are colostrum deprived or not, and the use of stress or virus exposure prior to challenge. It is my observation that neonatal calves do not respond to *P. haemolytica* vaccination and challenge the same as weanling cattle. Their immune responses are less intense, they are highly susceptible to low challenge doses, and individual animal variation is marked (Confer, unpublished data). Because pneumonic pasteurellosis is mainly a disease of weanling cattle, it would be desirable to conduct immunogenicity studies or potency tests in the older cattle. Use of weanling cattle is not without problems, however, they cost more to use and often have preexisting naturally acquired antibodies to *P. haemolytica* or can develop them prior to vaccination. Studies in my laboratory have been conducted with a transthoracic challenge model. This is an unnatural route of infection but has certain advantages over more conventional routes of challenge including rapidity of the challenge procedure, ease of quantifying lesions, and the ability to readily differentiate experimental from naturally acquired pneumonia. The infectious bovine rhinotracheitis virus/intratracheal or intrabronchial *P. haemolytica* challenge models are commonly used. These models have merit due to their natural route of infection and similarity to the natural disease in terms of clinical signs and lesions. Procedures for disease scoring varies among laboratories. Determination of a standard animal challenge model should help in comparison of data collected by various laboratories.

With the high cost of experimentation in cattle and society's increasing concern for animal welfare, it would be desirable to develop in vitro potency tests for non-living *P. haemolytica* biologicals. This brings me to the second issue. What are the important immunogens for inclusion in a *P. haemolytica* biological? If such a question could be answered with surety, then antigens could be quantified in vitro and compared to known standards determined in

immunogenicity studies. Unfortunately, the question cannot be answered with surety. Data indicate that surface antigens – particularly capsule, OMP, and iron-regulated proteins – and LKT are most likely the important immunogens. Immunity probably requires antibody to combinations of those immunogens. Because OMP stimulate homotypic immunity and capsules of various serotypes appear to be serotype specific, LKT may be of major importance for stimulating cross protection against various serotypes. Therefore, determination of the immunizing doses and relative concentrations of the important immunogens must be a major consideration of future *P. haemolytica* research.

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